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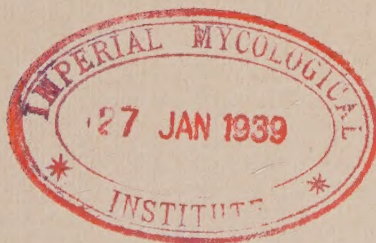
Differential Reaction of Apple Varieties To *Gymnosporangium* *Juniperi-Virginianae*

By GEORGE L. McNEW

AGRICULTURAL EXPERIMENT STATION
IOWA STATE COLLEGE OF AGRICULTURE
AND MECHANIC ARTS

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BOTANY AND PLANT PATHOLOGY SECTION



RESEARCH BULLETIN 245
DECEMBER, 1938
AMES, IOWA

December, 1938

Research Bulletin 245

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SUMMARY

Most of the collections of *Gymnosporangium juniperi-virginianae* from seven states and different localities in Iowa produced aecidia on Bechtel's Crab, Wealthy, Jonathan and Rome Beauty; flecks with or without spermogonia on York Imperial, Tolman, Ben Davis, Maiden Blush, Oldenburg, Turley and Grimes Golden and flecks on Delicious and Northwestern Greening.

The collections could be classified into eight groups on the basis of the differential reactions of certain of these varieties. Four of these groups were considered as possible parasitic races with the following differential reactions: The first one produced aecidia on Tolman and York Imperial; the second caused a diffuse spreading fleck on York Imperial and defoliation of Jonathan after large aecidial sori had been produced; the third failed to infect the varieties Delicious and Northwestern Greening; and the fourth one defoliated Turley after producing spermogonia.

Some of the differential reactions were correlated with different degrees of development of the mycelium in the leaf tissues. All collections studied, with the possible exception of the noninvasive race on Delicious and Northwestern Greening, penetrated the epidermis and became inter- or intracellular in the palisade layer. The hyphae from collections that caused punctiform flecks on Ben Davis and Turley had collapsed and were surrounded by injured cells of the palisade parenchyma. The hyphae of collections that caused large diffuse flecks on these varieties became established in the spongy parenchyma, even though the palisade layer was injured. The collections that produced spermogonia in the diffuse flecks on Ben Davis caused pronounced hypertrophy of cells in the spongy parenchyma. The hypertrophied cells in contact with the spermogonia collapsed before its maturation.

The mesophyll cells underwent hypertrophy and hyperplasia before spermogonia were formed in all varieties except Maiden Blush. The mycelium developed in the palisade layer of this variety and produced spermogonial initials, but cells of the spongy parenchyma appeared sensitive to the haustoria.

Differential Reaction of Apple Varieties to *Gymnosporangium* *Juniperi-Virginianae*¹

BY GEORGE L. McNEW²

Specialization in the cedar-apple rust fungus, *Gymnosporangium juniperi-virginianae* Schw. for its aecidial hosts was observed by Bliss (2) and Crowell (7). Since these investigators did not describe the distribution and host range of the races exhibiting specialization, studies were undertaken to ascertain the reaction of 12 varieties of apples to rust collections from different states. The purpose of this bulletin is to present evidence of specialization for several of these 12 varieties and to describe the histological changes of invaded leaf tissues that might explain some of the differential reactions observed.

MATERIALS AND METHODS

Bechtel's crab and the following varieties of apples (listed in order of increasing resistance to rust) were used as differential hosts: Wealthy, Jonathan, Rome Beauty, York Imperial, Tolman, Ben Davis, Maiden Blush, Oldenburg (Duchess), Turley, Grimes Golden, Delicious and Northwestern Greening. These varieties were selected because their reaction to rust ranged from complete susceptibility to high resistance and they had been reported (compilations by Bliss (2, 3) and Crowell (8)) to react differently to cedar-apple rust in different states.

Two-year-old trees of these varieties were obtained from a reliable wholesale nursery and planted in composted soil in 4-gallon glazed jars early in March. About five trees of each variety were planted and each tree was pruned back so that seven to nine terminal buds developed. The leafy shoots from these buds were about 8 to 12 inches long at the time of inoculation. Rust was collected from red cedars, *Juniperus virginiana* L., in different sections of Iowa late in April or early in May. Several twigs that supported large galls were cut, wrapped in wax paper, packed in moist sphagnum and held at 5° to 10° C. Collections treated in this fashion remained capable of producing sporidia for at least 4

¹Project 478 of the Iowa Agricultural Experiment Station.

²The author expresses his appreciation to Dr. I. E. Melhus for proposing the problem and for assistance in preparing the manuscript. He is also indebted to D. E. Bliss and C. J. Nusbaum for technical advice and to A. Berg, C. E. Temple, R. Bissey, O. H. Elmer, D. Cation, J. M. Hamilton, H. W. Anderson and H. B. Groves for supplying inoculum from states other than Iowa.

weeks. Many of the collections mailed from other states, however, gave poor sporulation after storage because of desiccation enroute or invasion by other fungi such as a species of *Monilia*.

Usually inoculum was obtained from a single large gall in each collection, but in a few instances two smaller galls from the same tree were used. The red cedar branch that supported the gall was trimmed into a sharpened handle about 2 inches long and forced into the small end of the cork stopper to a wide-mouth bottle. The bottle was filled with water and the cork placed in position so that the gall was suspended in water. After the spore horns had expanded thoroughly (1 to 3 hours), most of the water was poured off, and the gall was suspended over water in the stoppered bottle until the teleutospores germinated promycelia freely ($\frac{1}{2}$ to 2 hours). Production of sporidia was induced by pouring out the remaining water and admitting air around the loosened stopper. As soon as the spore horns became light rust-brown in color they were used as inoculum, provided that sporidia were abundant. Several collections from states other than Iowa (table 1) were tested even though they produced few sporidia.

All inoculations were made under greenhouse conditions. Each leafy shoot was thoroughly atomized with tap water so that there would be some free water on the upper surface. A sporidia-coated spore horn was pinched from the gall with forceps and gently smeared over the upper surface of each leaf on a shoot. A moistened 10-pound paper bag was then tied over the inoculated shoot to prevent contamination with other cultures on adjacent shoots. All equipment, such as forceps, was placed in 80 percent alcohol for at least 10 minutes and rinsed in distilled water after inoculating with each collection. As a check on the technique employed, two or three shoots of each variety were sprayed with distilled water and covered with a bag after all inoculations had been made. No sori developed on these uninoculated leaves.

High humidity was maintained in the greenhouse for 12 to 24 hours following inoculation, then the room was allowed to dry out gradually for 12 hours. The paper bags were removed 3 days later, and precautions were taken to prevent water from touching the leaves during the ensuing week. The sporidia probably were non-viable at the end of this period since Reed and Crabill (30) have shown that they cannot survive desiccation for more than 5 or 6 days. The inoculated leaves were examined for flecking and spermogonia immediately before the trees were moved from the greenhouse about 4 weeks after inoculation. They were examined for aecidia 60 to 90 days after inoculation and again for infection and the degree of defoliation later in the summer.

In 1935, sori on one of the three or four youngest infected leaves of each variety were taken for histological examination. Leaf tissue containing infection centers was killed and fixed in chromo-acetic-

TABLE 1. REACTION OF BECHTEL'S CRAB AND NINE VARIETIES OF APPLES TO *G. JUNIPERI-VIRGINIANAE* COLLECTED IN 23 DIFFERENT LOCALITIES IN 1933.

No.	Region	Rust collection		Reaction of different varieties ¹									
		Bechtel's Crab	Wealthy	York Imperial	Tolman	Ben Davis	Maiden Blush	Oldenburg	Grimes Golden	Delicious	N. W. Greening		
1	Mills Co., Ia.	Ae	Ae	Fl	Fl	—	Sp	Sp	Sp	O	O	O	O
2	Pottawattamie Co., Ia.	Ae	Ae	Fl	Fl	—	Sp	Sp	Sp	O	O	O	O
3	Harrison Co., Ia.	—	Ae	—	Ae	—	Sp	O	Sp	O	O	O	O
4	Carroll Co., Ia.	—	Ae	—	O	—	—	O	Sp	O	O	O	O
5	Pottawattamie Co., Ia.	—	Ae	O	—	—	—	O	Fl	O	O	O	O
6	Greene Co., Ia.	Ae	Ae	Fl	O	—	—	—	Sp	O	O	O	O
8	Story Co., Ia.	Ae	Ae	O	O	—	—	O	Fl	O	O	O	O
10	Clarke Co., Ia.	—	O	O	O	—	—	O	Fl	O	O	O	O
20	Cass Co., Ia.	Ae	Ae	O	O	Sp	O	O	Fl	O	O	O	O
21	Montgomery Co., Ia.	—	Ae	O	O	Fl	O	O	Fl	Fl	O	O	O
22	Van Buren Co., Ia.	—	Fl	O	O	Fl	Sp	O	Sp	O	O	O	O
24	Lee Co., Ia.	Ae	Ae	Fl	Fl	O	O	O	Fl	O	O	O	O
25	Backbone Park, Ia.	Ae	Ae	Fl	O	Fl	—	—	Fl	Fl	O	O	O
7	Morgantown, W. Va.	Ae	Sp	Ae	Ae	—	O	Fl	Fl	O	O	O	O
9	Durham, N. H.	O	O	O	O	—	O	O	O	O	O	O	O
11	Manhattan, Kan.	O	O	O	O	—	O	O	O	O	O	O	O
12	Maryland	Ae	Ae	Fl	Fl	—	O	Fl	Sp	O	O	O	O
13	Southern Illinois	—	Ae	Fl	Fl	—	O	O	O	O	O	O	O
14	Poughkeepsie, N. Y.	O	O	O	O	—	—	—	O	O	O	O	O
16	Linden, Mo.	—	Ae	O	O	Sp	—	—	Fl	Fl	O	O	O
17	Kansas City, Mo.	—	O	O	O	Fl	—	—	Fl	Fl	O	O	O
18	Platte Co., Mo.	Ae	Ae	Sp	O	Sp	—	—	—	O	O	O	O
15	Winchester, Va.	—	O	—	—	—	—	—	—	—	—	—	—

¹Legend for reaction: Ae = aecidia formed; Sp = spermogonia developed; Fl = yellow fleck; O = no visible symptom; — = no test made or branch injured from other causes.

formalin mixture for 48 hours, washed in running water, dehydrated with butyl alcohol according to the method described by Nusbaum (28) and embedded in paraffin. Sections were cut 5 to 7 μ thick and stained with Flemming's triple stain, a fast green modification of the triple stain or a cotton blue-orange G stain. The sections were over-stained with cotton blue in lacto-phenol, rapidly passed through alcohol and counterstained with orange G in absolute alcohol and xylol before they were differentiated in xylol. The cotton blue was found to be an excellent general stain for mycelium which has dense cytoplasm (fig. 5, B). However, depleted mycelium does not stain since the mycelial wall is refractory to cotton blue.

EXPERIMENTAL RESULTS

EXPERIMENT OF 1933

Inoculations were made the first week of May in 1933 with rust collected in the various localities listed in table 1. Since some of the trees died or lost most of their leaves before they were examined for infection on June 20, data were not obtained on all host-collection combinations. Additional observations were made on July 21 to detect any aecidial cups which might have developed. The most advanced stage of development observed for each collection of rust is reported in table 1.

TABLE 2. REACTION OF 12 APPLE VARIETIES TO COLLECTIONS OF *G. JUNI-PERI-VIRGINIANA* FROM 36 DIFFERENT LOCALITIES IN IOWA.

Rust collection		Reaction of different varieties ¹											
No.	Locality in Iowa	Wealthy	Rome Beauty	Jonathan	York Imperial	Tolman	Ben Davis	Maiden Blush	Oldenburg	Turley	Grimes Golden	Delicious	N. W. Greening
1	State Center	Ae ¹	Ae ¹	Ae ¹	F1 ¹	F1 ¹	F1 ²	Sp	Sp	—	—	F1 ¹	F1 ²
2	Osceola	Ae ¹	Ae ¹	Ae ¹	F1 ¹	O	F1 ¹	Sp	Sp	F1 ¹	F1 ¹	F1 ²	F1 ²
3	Osceola	Ae ¹	Ae ¹ , F1 ²	Ae ¹	Sp	F1 ¹	F1 ²	Sp	Sp	F1 ¹	F1 ¹	O	F1 ²
4	Afton	Ae ¹	Ae ¹	Ae ¹	F1 ¹	O	Sp	Sp	Sp	F1 ¹	F1 ¹	F1 ²	F1 ¹
5	Creston	Ae ¹	Ae ¹	Ae ¹	F1 ¹	F1 ¹	Sp	Sp	Sp	Sp	Sp	F1 ¹	F1 ¹
6	Corning (E.)	Ae ¹	Ae ¹	Ae ¹	F1 ¹	F1 ¹	Sp	Sp	F1 ¹	Sp	F1 ²	F1 ¹	F1 ¹
7	Corning (W.)	Ae ¹	Ae ¹	Ae ¹	F1 ¹	Sp	Sp	Sp	F1 ¹	F1 ¹	Sp	F1 ¹	F1 ¹
8	Marshalltown	Ae ¹	Ae ¹	Ae ¹	F1 ¹	F1 ¹	F1 ²	F1 ¹	—	—	F1 ¹ + ²	F1 ¹	O
9	Malvern	Ae ¹	Ae ¹	Ae ¹	F1 ¹	F1 ¹	F1 ¹	Sp	F1 ¹	O	F1 ¹ + ²	F1 ¹	F1 ²
10	Council Bluffs (N)	Ae ¹	Ae ¹	Ae ¹	F1 ¹	F1 ¹	F1 ²	Sp	—	O	F1 ¹ + ²	F1 ¹	F1 ²
11	Belle Plaine	Ae ¹	Ae ¹	Ae ¹	F1 ¹	F1 ¹	F1 ²	Sp	F1 ¹	F1 ¹	Sp	F1 ¹	O
12	Glenwood	Ae ¹	Ae ¹	Ae ¹	F1 ¹	F1 ¹	F1 ²	Sp	F1 ¹	F1 ¹	F1 ¹ + ²	F1 ¹	F1 ²
13	Missouri Valley	Ae ¹	Ae ¹	Ae ¹	Sp	O	F1 ²	Sp	Sp	F1 ¹	F1 ¹	F1 ¹	F1 ²
14	Keystone	Ae ¹	Ae ¹	Ae ¹	F1 ¹	F1 ¹	F1 ²	F1 ¹	F1 ¹	F1 ¹	F1 ¹ + ²	F1 ¹	F1 ²
15	Magnolia	Ae ¹	Ae ¹	Ae ¹	F1 ¹	O	F1 ²	Sp	Sp	F1 ¹ , O	F1 ¹	—	F1 ¹
16	Logan	Ae ¹	Ae ¹	Ae ¹	F1 ¹	F1 ¹	F1 ²	Sp	F1 ¹	F1 ¹	F1 ¹	F1 ¹	F1 ²
17	Denison	Ae ¹	Ae ¹	Ae ¹	F1 ¹	F1 ¹	F1 ²	Sp	Sp	F1 ¹	F1 ¹	O	F1 ²
18	Carroll	Ae ¹	Ae ¹	Ae ¹	F1 ¹	F1 ¹	—	F1 ¹	F1 ²	F1 ¹	F1 ¹	F1 ¹	F1 ²
19	Scranton	Ae ¹	Ae ¹	Ae ²	F1 ²	Sp	F1 ²	Sp	Sp	O	F1 ¹	F1 ²	F1 ²
20	Cedar Rapids	Ae ¹	Ae ¹	Ae ¹	F1 ¹	F1 ¹	F1 ²	Sp	Sp	F1 ¹	F1 ¹ + ²	F1 ¹	F1 ²
21	Jefferson (E.)	Ae ¹	Ae ¹	Ae ¹	F1 ¹	F1 ¹	F1 ²	F1 ¹	Sp	—	Sp	F1 ¹	F1 ²
22	Cedar Rapids	Ae ¹	Ae ¹	Ae ¹	F1 ¹	F1 ¹	F1 ²	Sp	Sp	F1 ¹	Sp	F1 ¹	O
23	Palisades St. Park	Ae ¹	Ae ¹	Ae ¹	F1 ¹	F1 ¹	F1 ²	Sp	F1 ¹	F1 ¹	Sp	F1 ¹	O
24	Anamosa	—	Ae ¹	Ae ¹	F1 ¹	F1 ¹	F1 ²	Sp	Sp	F1 ¹	O	O	O
25	Cascade	Ae ¹	Ae ¹	Ae ¹	F1 ¹	F1 ¹	F1 ²	Sp	—	F1 ¹	F1 ¹ + ²	F1 ²	O
26	Dubuque	Ae ¹	Ae ¹	Ae ¹	F1 ¹	F1 ¹	F1 ²	Sp	—	F1 ¹	Sp	F1 ¹	F1 ²
27	Holy Cross	Ae ¹	Ae ¹	Ae ¹	F1 ¹	F1 ¹	F1 ²	Sp	—	F1 ¹	Sp	F1 ¹	F1 ²
28	Luxemburg	Ae ¹	Ae ¹	Ae ¹	F1 ¹	F1 ¹	F1 ²	Sp	—	O	F1 ²	F1 ¹	F1 ²
29	Guttenburg	Ae ¹	Ae ¹	Ae ¹	F1 ¹	F1 ¹	F1 ²	Sp	—	O	F1 ²	F1 ²	F1 ²
30	Clayton Center	Ae ¹	Ae ¹	—	F1 ¹	F1 ¹	F1 ²	Sp	—	F1 ¹	Sp	F1 ¹	O
31	Elkhart	Ae ¹	Ae ¹	Ae ¹	F1 ¹	F1 ¹	F1 ²	Sp	—	F1 ¹	—	F1 ²	O
32	West Union	Ae ¹	Ae ¹	Ae ¹	F1 ¹	F1 ¹	F1 ²	Sp	—	F1 ¹	F1 ²	F1 ¹	O
33	Waverly	—	Ae ¹	Ae ¹	F1 ¹	F1 ¹	F1 ²	Sp	—	F1 ¹	F1 ²	F1 ²	F1 ²
34	Shellrock	Ae ¹	Ae ¹	Ae ¹	F1 ¹	F1 ¹	F1 ²	Sp	—	F1 ¹	F1 ²	O	O
35	Allison	Ae ¹	Ae ¹	—	F1 ¹	F1 ¹	F1 ²	Sp	—	F1 ¹	Sp	F1 ¹	O
36	Hampton	—	Ae ¹	Ae ¹	F1 ¹	—	F1 ²	Sp	—	F1 ¹	F1 ¹ + ²	F1 ¹	F1 ²

¹Legend for reaction: Ae¹ = typical production of aecidia; Ae² = large diffuse sorus with delayed aecidial production; Sp = spermogonia formed; F1¹ = restricted yellow fleck; F1² = diffuse yellow fleck; O = no infection apparent; — = inoculated branch dead or defoliated by other agents.

The rust from West Virginia produced aecidia on the varieties Tolman and York Imperial as described by Bliss (2). There was a conspicuous swelling of the infected areas on June 20, and aecidia developed later. The aecidia developed more rapidly and were more numerous on the variety Tolman than on York Imperial. Aecidia were also produced on leaves of the variety Tolman inoculated with rust (no. 3) collected in western Iowa, 4 miles east of Logan in Harrison County. The York Imperial tree inoculated with this culture died from other causes. These data indicate that the strain of rust found in West Virginia also occurs in Iowa. It apparently is very common in West Virginia since it appeared in this test and in the one reported by Bliss (2). It may be rather local in Iowa and other midwestern states since rust collection no. 3 was the only one to produce aecidia on either Tolman or York Imperial.

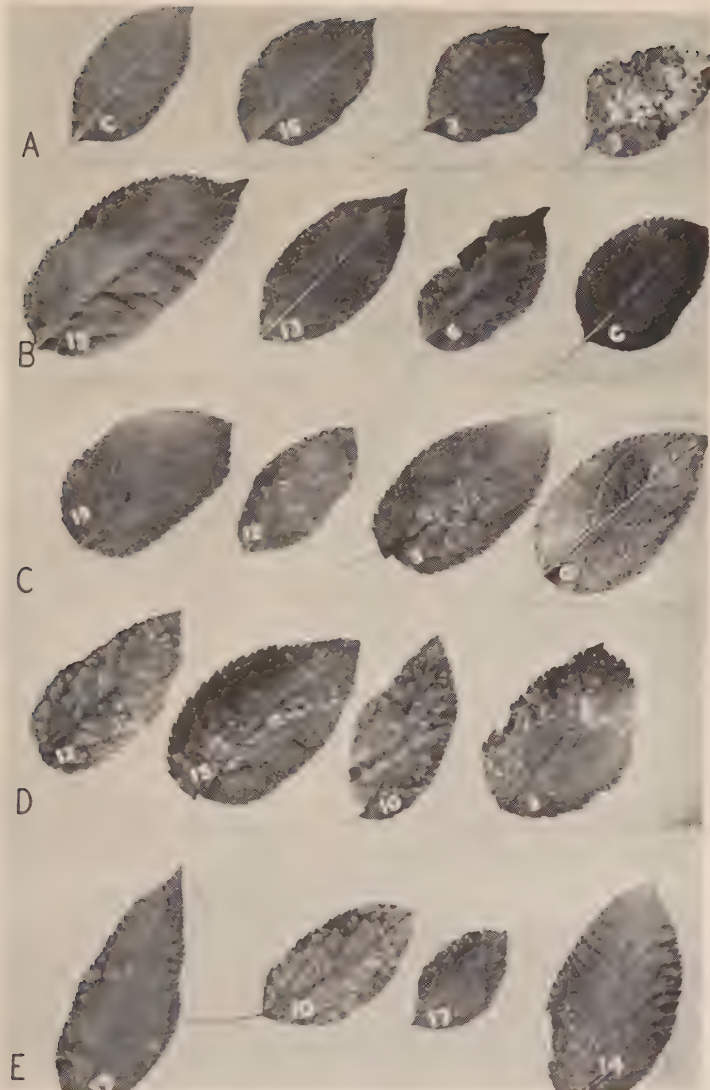


Fig. 1. Sori produced by different collections of *G. juniperi-virginianae* 36 days after inoculation. A and B. Turley: Most collections produced the yellow sori illustrated by 13 and 16; no. 15 (class 8) was practically non-invasive; class 4 (nos. 5 and 6) produced spermogonia and caused defoliation. C and D. York Imperial: Yellow flecks were usually produced by most collections such as nos. 5, 10 and 15; spermogonia were produced in similar flecks by nos. 3 and 13; class 2 (no. 19) caused an atypical diffuse type of chlorosis. E. Jonathan: Sori produced by collections nos. 10, 14 and 17 are typical of most collections, but class 2 (no. 19) produced exceptionally large sori, delayed production of spermogonia and aecidia and caused defoliation.

Red spiders injured the leaves so severely that flecking was recorded in table 1 only when it could be identified as distinctly due to the rust. Although some rust collections may have failed to infect the varieties Delicious and Northwestern Greening (see 1935 experiment), most of the negative results were recorded because it was impossible to differentiate between the injury caused by rust and red spiders.

This experiment indicated that symptoms other than production of aecidia might be used as differential characters. For instance, collections nos. 16, 17, 21 and 25 produced pronounced sori with necrotic centers on the variety Delicious, instead of the usual restricted yellow fleck. Furthermore, several collections produced spermogonia on the varieties Grimes Golden and Ben Davis while most collections merely caused a small fleck.

EXPERIMENT OF 1935

Since the 1933 experiment indicated that the West Virginia strain occurred in Iowa, the range of virulence and distribution of strains in Iowa were studied in experiments conducted in 1935. The sources of inoculum and the differential hosts used are listed in table 2. Rust was collected between April 20 and 30, and inoculum was applied April 23 to May 2. The inoculated leaves were examined for spermogonia and flecking on June 1, for aecidia on July 12 and for final effect of infection on Aug. 21. Many of the more severely infected leaves, however, had fallen by the latter date. The most extreme type of development observed for each host-parasite combination is listed in table 2.

Some of the rust collections from the western half of Iowa (nos. 2 to 21, excepting 8, 11, 14 and 20) appeared to be more virulent on some of the varieties than those from the eastern counties. Collections nos. 5 and 6 were the only ones that completely defoliated the variety Turley, and nos. 4, 5, 6 and 7 were the only ones that produced spermogonia on the variety Ben Davis. No. 7 was one of the two collections that produced spermogonia on the variety Tolman, and nos. 6 and 7 caused the severer type of flecking on the variety Delicious. Of the 20 collections from eastern and northeastern Iowa, 10 failed to infect the variety Northwestern Greening, and three failed on the variety Delicious. The West Virginia strain was not collected in 1935. Rust collection no. 16, taken from the same tree as no. 3 of the 1933 experiment, failed to produce aecidia on either York Imperial or Tolman, as did rust collected from 11 other trees within a radius of 70 miles from this spot.

In order to determine whether the differential reactions observed in 1935 were associated with differences in the relationship of host and parasite cells, paraffin sections were prepared from several sori of each type on the different varieties. The pathological an-

atomy associated with differences in the macroscopic appearance of sori is described in the next section. The first part of the section is devoted to a description of the most common type of reaction for each variety, which is referred to in subsequent discussion as the "typical reaction" for each variety. Sori produced by the rust collections which showed specialization for certain varieties are described in the second part of the section.

MACROSCOPIC AND HISTOLOGICAL APPEARANCE OF SORI

TYPICAL REACTION OF EACH VARIETY

The types of reaction shown by the different varieties to a large percentage of the rust collections are described briefly below.

WEALTHY, ROME BEAUTY AND JONATHAN

These three most susceptible varieties supported aecidia in severely hypertrophied leaf tissue. The reaction of these extremely susceptible varieties (figs. 1, E and 2, E) is so well known that it needs no description. The aecidial cups on Wealthy opened 67 to 75 days after inoculation and those on Rome Beauty and Jonathan about 10 days later. Although aecidia of some collections on Rome Beauty did not mature until 2 weeks after others, no attempt was made to separate races on the basis of time required for aecidia to mature, as has been done by Crowell (7) for strains specialized for different species of *Malus*.

Histological studies of the invaded tissue showed little evidence of antagonism between host and parasite (fig. 4, E) other than hypertrophy and hyperplasia of host cells. Although the appearance of susceptible tissues invaded by *G. juniperi-virginianae* has been described (7, 28, 30), the following general observations of collections no. 21 on Wealthy, no. 12 on Rome Beauty and no. 30 on Jonathan are recorded for comparison with the less susceptible varieties. In these three varieties, the cuticle and upper epidermis were penetrated, the palisade parenchyma traversed and the spongy parenchyma invaded for considerable distances by mycelium which was still functional at the periphery of the sorus. Although filamentous haustoria were present in many cells of the mesophyll and epidermis, the spongy parenchyma cells were the only ones to show pronounced hypertrophy and hyperplasia. Most of these cells were not appreciably injured (figs. 5, B and 6, E) and appeared normal except for their shape and the inclusion of brown oil-drop-like bodies and other smaller bodies that stained with safranin. Some of the cells of the palisade layer were injured, particularly in Rome Beauty and Jonathan, but not sufficiently to prevent maturation of spermatogonia. At the time material was collected for sectioning, Wealthy contained mature aecidia with binucleate aecidiospores, but the aecidia on the other two varieties were not so well developed (fig. 4, E and F).

YORK IMPERIAL

The typical infection on York Imperial consisted of a small definite fleck (fig. 1, D) surrounded by a narrow band of yellow tissue. Although collections nos. 3 and 13 developed sufficiently to produce abortive spermatogonia in some of these flecks before the host tissue collapsed, the reaction was not sufficiently consistent to be considered differential.

The cuticle and upper epidermis had been penetrated directly (collection no. 7) and the palisade layer traversed by strong, intercellular hyphae. The mycelium became established in the mesophyll, and runner hyphae grew laterally for 15 to 30 cells. Haustoria penetrated the host cells, and the mycelium appeared to be functional except where it was in contact with injured cells of the spongy parenchyma and lower epidermis. Cells of the palisade layer showed no hypertrophy and those of the spongy parenchyma very slight hypertrophy and no hyperplasia. The contents of the spongy parenchyma cells a few microns back of the growing tips of the mycelium were granular and dying. The upper layer of palisade cells was severely injured, and the epidermal cells over the injured areas had collapsed (similar to Turley, fig. 6, F). Apparently the pathogen's development was limited only by the injury to the host.

TURLEY

Several intergrading reactions were shown by Turley, which ranged from a small fleck consisting of yellow tissue, through a large irregular yellow area 2-6 mm. in diameter with a necrotic center, to a severe chlorosis and necrosis that caused defoliation. The typical infection, as illustrated by collections nos. 13 and 16, fig. 1, A and B, consisted of a large, definite yellow spot. The single spermatogonial cushions initiated in some of the sori never matured.

The cuticle and epidermis were penetrated directly (collections nos. 11 and 3), and the mycelium apparently spread laterally in the epidermis since fragments could be identified in collapsed epidermal cells. Inter- and intracellular hyphae penetrated the palisade parenchyma which was severely injured (fig. 6, F). The mycelium grew intercellularly in the second layer of palisade cells and established filamentous haustoria in many of them. Although mycelium was present in the spongy parenchyma, few haustoria were present and the cells exhibited only slight hypertrophy and no hyperplasia.

BEN DAVIS

The typical sorus consisted of a rather large diffuse fleck. Most of the invaded tissue was yellow with very little necrosis. The mycelium (collection no. 23) became established in the spongy parenchyma after the epidermis had been penetrated and the palisade layer traversed by inter- and intracellular hyphae. Some of



Fig. 2. Sori produced by different collections of *G. juniperi-virginianae* 36 days after inoculation. A and B. Delicious: The small yellow flecks produced by nos. 5, 7, 12 and 13 were typical of most collections; 3 and 17 were practically noninvasive. C and D. Northwestern Greening: Yellow and necrotic flecking caused by different collections. E. Rome Beauty: Sori and spermogonia produced by different collections. The atypical appearance of no. 5 is due to the age of the leaf at time of inoculation.

these hyphae apparently died shortly after penetrating the palisade layer. Mycelium in the spongy parenchyma was exceptionally stout (fig. 6, C), and even though haustoria penetrated the cells no runner hyphae were sent through the mesophyll. Spongy parenchyma cells in contact with the hyphae became granular and contained oil droplets but were not injured so severely as those in York Imperial or Maiden Blush (fig. 6, J). Large sections of the palisade layer and adjacent epidermis had collapsed.

NORTHWESTERN GREENING

The typical sorus consisted of a definite fleck on this variety (collections nos. 2, 3 and 13; fig. 2, C and D). The flecks produced by most collections were yellow, but several collections such as nos. 5 and 6 produced abortive spermogonia and necrosis in the center of the yellow zone. Although these two collections developed to a more advanced stage, they are not classified as a distinct group, because many of the sori contained no spermogonia.

Mycelium (collection no. 6) penetrated the palisade layer inter- or intracellularly and spread through the spongy parenchyma. In most sori, cells of the palisade layer were injured and the mesophyll collapsed to give a necrotic area, but in the less severely injured sori the mycelium became very stout and sent haustoria into the cells which underwent pronounced hypertrophy and some hyperplasia. Mycelium in some of these hypertrophied areas was aggregated into spermogonial cushions. Most of the hypertrophied palisade cells apparently collapsed before the spermogonia matured.

DELICIOUS

The typical infection consisted of a single fleck containing yellow tissue (collections nos. 5 and 12; fig. 2, A and B). Young leaves inoculated with collections nos. 7 and 13 showed a severer type of flecking than those inoculated with other collections. These two collections did not appear to be distinctly different from the others, however, since older leaves inoculated with them produced the typical light flecks. Mycelium (collection no. 7) penetrated the palisade layer inter- and intracellularly from the epidermis. Mycelium grew intercellularly in the palisade layer and sent many slender haustoria into the cells after establishment, even though four to six cells to either side of the point of penetration were injured. The chloroplasts in the invaded area degenerated. Very few hyphae were present in the spongy parenchyma.

GRIMES GOLDEN

Although most collections produced a definite fleck on this variety (nos. 5 and 13 of fig. 3, B), several collections such as no. 6 caused a diffuse type of chlorosis on younger leaves. This diffuse flecking is not considered a suitable differential character, because older leaves showed typical definite flecks, many collections produced both types of flecking (collection no. 12, fig. 3, B), and there was no distinct histological difference between the two types. About half

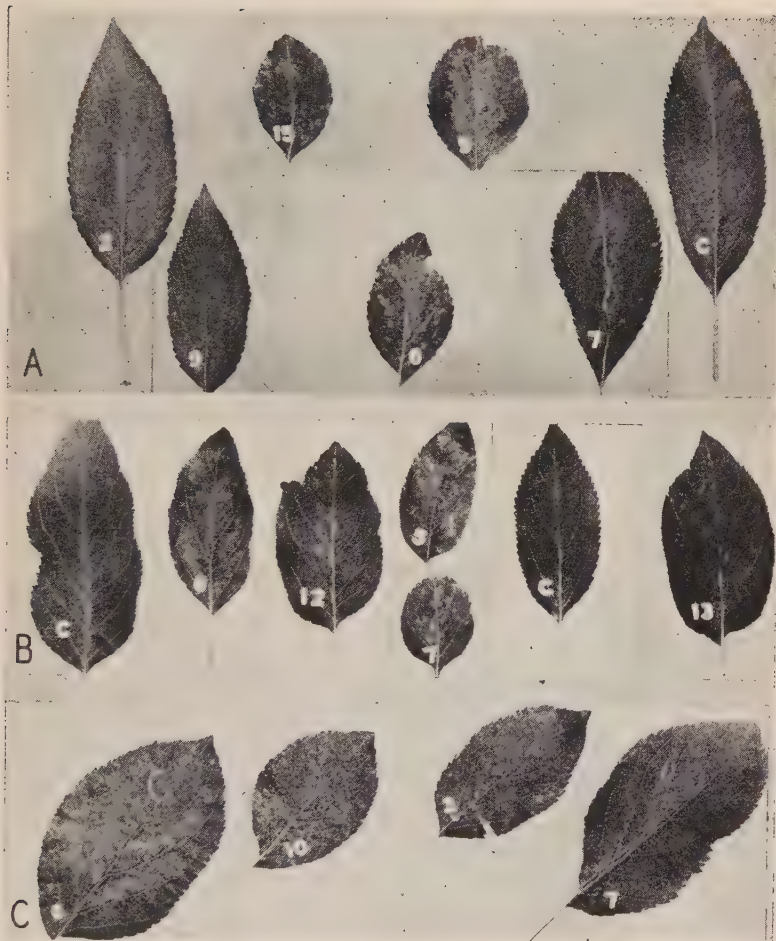


Fig. 3. Sori produced by different collections of *G. juniperi-virginianae* 36 days after inoculation. A. Ben Davis: No. 15 caused the typical diffuse type of fleck; nos. 2 and 9 (class 6) caused punctiform flecks; nos. 5, 6 and 7 (class 7) produced spermogonia in the diffuse flecks. B. Grimes Golden: Typical flecks caused by nos. 5 and 13; a few collections produced diffuse flecking as in no. 6, and others produced spermogonia as in no. 7. C. Oldenburg: Yellow flecks ordinarily produced (the minute specks were caused by red spiders).

of these collections produced abortive spermogonia in some of the flecks. Although collection no. 24 is recorded as noninvasive on this variety this reading is open to question, since only three leaves remained on the branch at the time of reading.

Final development of the mycelium was about the same in an intense, definite fleck (caused by collection no. 7) and a more diffuse fleck (caused by collection no. 33). The mycelium developed inter-

cellularly in the palisade layer for considerable distances. Runner hyphae developed profusely (figs. 5, E and 6, A) in the spongy parenchyma, but few haustoria penetrated the cells, which showed very slight hypertrophy. Many of the runner hyphae were depleted, indicating that they were not obtaining adequate nourishment from the host cells. Absence of protoplasm in the mycelium could not be attributed to the transfer of cell contents to spermogonia and aecidia [a phenomenon described by Liu (18, 19) and Nusbaum (28) for *Wealthy*], since there were no spermogonia in most of the sori examined. Furthermore, the intermingling of functional and depleted hyphae (fig. 6, A) suggests that certain hyphae failed to become established in the host. There was no indication of hypersensitivity, since the mesophyll cells appeared normal, and cells of the palisade layer were not injured excessively.

MAIDEN BLUSH

The typical infection consisted of a definite fleck (similar to those illustrated for Oldenburg in fig. 3, C), some of which supported abortive spermogonia. Since both flecks and spermogonia were produced by different sporidia in the same collection, this reaction is not considered differential. Sori containing abortive spermogonia from collection no. 25 were used for histological studies. Inter- and intracellular mycelium developed profusely in the palisade layer and aggregated into cushions, a few of which developed into spermogonia before dying. These abortive spermogonia were produced in tissue which showed practically no hypertrophy (fig. 5, C), a phenomenon which was not observed in other varieties. Cells at the point of penetration were severely injured (fig. 6, I), but those in contact with spermogonia were not injured sufficiently to prevent maturation of the spermogonia as was observed in Ben Davis and Turley.

Mycelium grew throughout the mesophyll, but most of the older hyphae were depleted. Although the depleted mycelium appeared similar to that in Grimes Golden, the two hosts differed in that cells of the spongy parenchyma in Maiden Blush had granular shrunken protoplasts. At the periphery of the sorus, many of the hyphae in contact with dead host cells had collapsed, although the remainder of the mycelium was normal in appearance. The remains of a haustorium could be identified in one cell in which both the host cell and parasite had collapsed (fig. 6, J, a). Since more recently penetrated cells nearer the edge of the sorus were severely injured, and the mycelium had not collapsed (fig. 6, J, b), it is concluded that the host was hypersensitive to the haustoria, and injury to the mycelium was a secondary effect.

The fact that many of the runner hyphae throughout the sorus were depleted suggests that the failure of the haustorium to establish contact with a functional host cell prevented nourishment of the pathogen. Contents of the mycelium may have been trans-

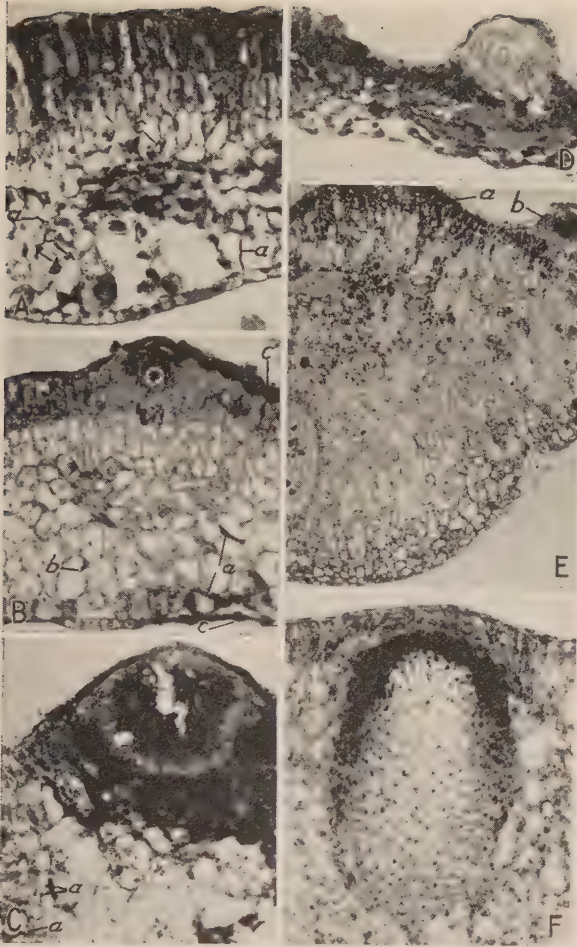


Fig. 4. Histological appearance of sori on Turley, Wealthy and Jonathan. A. Typical reaction of Turley (collection no. 11): Slight hypertrophy, severe injury to the cells of the palisade, depleted hypha (a), active hypha (b) and haustoria (c). This is the most advanced stage attained by this collection. x156. B. Spermogonium produced in sori on Turley by class 4 (collection no. 5): palisade layer scarcely injured except for trace of injury appearing at (c); inter-cellular hyphae (a) and haustoria (b) are functional. x156. C. A more advanced stage of the injury initiated at (c) in B: The host cells around the spermogonium have collapsed thereby depriving it of nourishment; mycelium at (a) appears to be functional. x156. D. Final collapse of Turley caused by class 4: Abortive spermogonia in necrotic tissue. x180. E. General details of infection on the susceptible host, Wealthy: Some palisade cells at (a) are injured but spermogonia develop in less severely injured sector at (b); hypertrophied spongy parenchyma cells adjacent to the mature aecidium are injured very little. x46. F. Immature aecidium on Jonathan separated from upper epidermis by a layer of crushed palisade cells; stained with cotton blue and orange G. x173.

ferred to the spermogonia as described by Liu (19) for more susceptible varieties. However, the failure of both spermogonia and mycelium to continue development might be attributed to failure of the haustoria to maintain suitable contact with the host.

OLDENBURG

Flecks similar to those on Maiden Blush (fig. 3, C) were produced, and abortive spermogonia developed in most of the sori. The material collected for histological studies was found to be unsatisfactory beyond showing that mycelium developed in the mesophyll.

TOLMAN

Definite flecks similar to those produced on York Imperial developed on Tolman. Of the atypical reactions observed, several collections failed to infect the inoculated leaves, but these readings are ignored, since only a few leaves were available for observation. These particular cultures and two others that produced a few sori were inoculated on leaves of small leafy spurs, since there was an insufficient number of leafy branches of this variety for each collection to be tested.

Histological symptoms of different sori (collection no. 10) differed, although their macroscopic appearance was similar. The epidermis and palisade layers were invaded in the same fashion, but further development of the mycelium caused one of two changes. In some sori there was abundant development of intercellular mycelium in the palisade layer and pronounced hypertrophy of the surrounding cells. Few hyphae extended into the spongy parenchyma, and in most infection centers the cells of the mesophyll were killed. In other sori the primary invasive hyphae penetrated the palisade layer and sent many hyphae through the spongy parenchyma. Many of the runner hyphae in the spongy parenchyma were depleted.

DESCRIPTION OF DIFFERENTIAL REACTIONS

Some of the rust collections did not produce the typical reactions, described above, on all varieties. These collections may be classified into eight groups on the data presented in tables 1 and 2. The first four of these groups are considered to be potential parasitic races. In suggesting this differentiation into races a conservative attitude should be assumed, since the accuracy of these observations is limited by at least three factors. In the first place, the experiments could not be repeated with a given set of collections, since *G. juniperi-virginianae* has no autoecious cycle on either host. It was necessary, therefore, to make fresh collections for each inoculation, and identical material was not always obtained from a given area. In the second place, leaves of different ages on a branch react differently to the same rust collection. Frequently the differences between virulent and avirulent strains were obscured

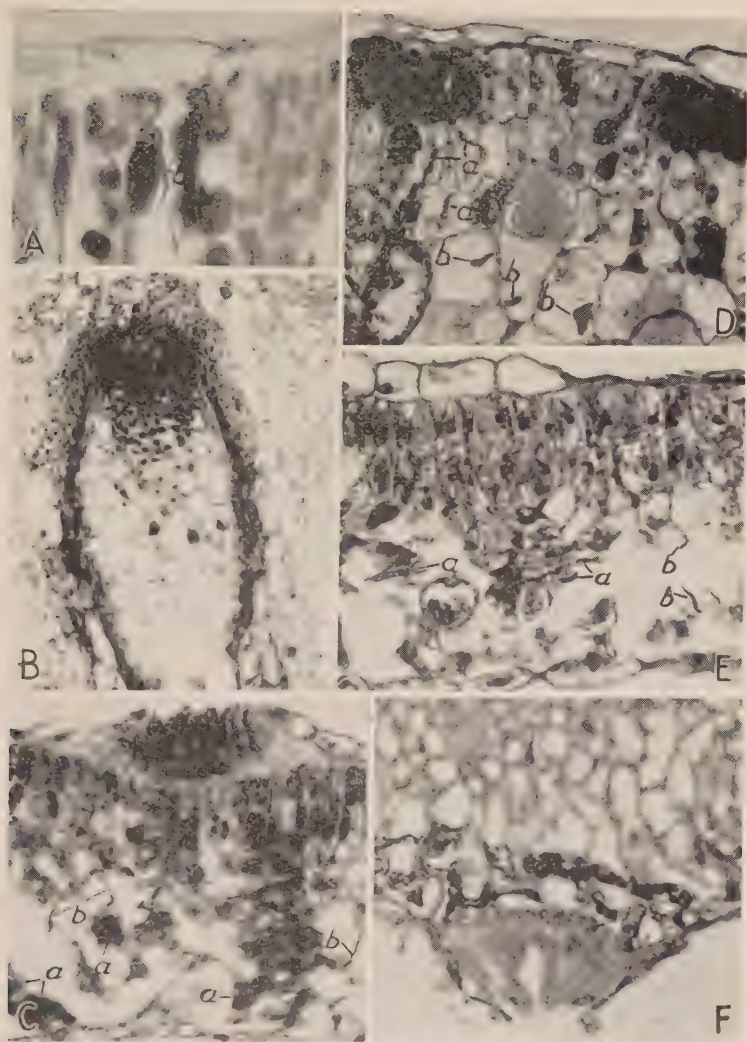


Fig. 5. Histological appearance of sori on Turley, Wealthy, Maiden Blush, Grimes Golden and Ben Davis. A. Collection no. 15 (class 8) on Turley: The remains of a collapsed hypha are shown at (b) among the injured palisade cells. x594. B. Accidium on Wealthy stained with cotton blue and orange G. x135. C. Abortive spermogonium on Maiden Blush: No hypertrophy of mesophyll cells, severe injury of cells at (a) and collapsed hyphae at (b). x222. D. Injury to cells of the palisade layer in Grimes Golden: Mycelium at (a) and haustoria at (b) became established in the spongy parenchyma before cells of the palisade layer collapsed. x414. E. Runner hyphae at periphery of sorus in Grimes Golden: Many of the hyphae are depleted at (b) but some functional ones remain at (a). x234. F. Collapsed hypertrophied cells at base of spermogonium of class 7 on under surface of Ben Davis. x324.

by partial failure of the virulent strain to infect the older leaves on the branch. Finally, the different sporidia from a collection often produced different types of sori, even on the same leaf (collections nos. 21 and 28 on York Imperial and several collections on Grimes Golden in 1935). The heterogeneity of these collections may be attributed to a segregation of Mendelian factors for virulence into the different sporidia produced by a heterozygous teleutospore. Liu (18) and Miller (23) have shown that *G. juniperi-virginianae* has a sexual cycle comparable to that described by Craigie (4, 5, 6) for other rusts. It follows that factors for virulence may segregate in the teleutospore and recombine in the aecidium as described by Newton *et al.* (26, 27) for *Puccinia graminis* (Pers.) Erikss.

The differential reactions of the eight parasitic groups recognized in this bulletin are described below.

Class No. 1. Collections of this group have the ability to produce spermogonia and aecidia on York Imperial and Tolman. The identity of this class is considered to be definitely established, since it has been observed on three different occasions (West Virginia collection no. 7 and Iowa collection no. 3 of the 1933 experiments and Bliss (2)). Waite (33) apparently recognized this class in the field as early as 1905. It is probably responsible for the severity of rust on York Imperial in Virginia (30) and West Virginia (13). No histological studies were made since it was not included in the 1935 experiment.

Class No. 2. The single collection (no. 19 of 1935 experiment), differentiated by its ability to produce a diffuse spreading type of flecking on York Imperial and large aecidial sori on Jonathan, is considered to be a distinct class. Further evidence of a differential reaction on Jonathan consisted of delayed production of spermogonia and aecidia in the large sorus (fig. 1, E) and ultimate defoliation of this variety. Only 2 of the 12 inoculated leaves remained on the tree by August and they fell off when touched. Leaves infected by other rust collections were firmly attached to the branches at this time. Collections nos. 21 and 28 may be heterozygous for the genetical factors determining this type of virulence, since they caused considerable defoliation of Jonathan and produced sporidia that caused diffuse, as well as definite, flecks on York Imperial.

This collection caused less severe injury to cells of the palisade layer in York Imperial (fig. 6, B) than did the typical ones described above. The palisade layer was penetrated by a stout primary invasive hypha that reached the spongy parenchyma and sent out a few short runner hyphae. The mycelium appeared to be dead at the time the sori were collected since it was partially collapsed and had a tendency to stain with safranin. Although no haustoria were observed, it was impossible to determine whether the



Fig. 6. Camera lucida drawings of infected leaf tissue. A. Typical infection on Grimes Golden: Depleted runner hyphae (a), active hyphae (b) and haustorium in epidermal cell (c). B. Dead primary hyphae (a) of collection no. 19 on York Imperial. C. Typical infection in spongy parenchyma of Ben Davis (palisade layer infected as shown in D): Slightly injured cells (a), stout mycelium (b), haustoria (c). D. Infection in palisade layer of Ben Davis by collection no. 7: Dead primary invasive hypha (a) surrounded by host cell, functional invasive hypha (b) and filamentous haustorium in slightly injured host cell (c). E. Haustorium in hypertrophied cell of Wealthy. F. Typical fleck on Turley: Primary invasive hypha (a) and haustoria (b) in injured cells of palisade layer. G. Punctiform fleck on Ben Davis caused by collection no. 2: Remains of hypha (a) in area of collapsed cells. H. Hypertrophied cells in Ben Davis (collection no. 7): Spermatogonia (fig. 5, F) were borne on under side of leaf in such palisade-like tissue. I and J. Typical infection on Maiden Blush: Cells of palisade layer are injured but mycelium develops abundantly and penetrates the spongy parenchyma; haustoria cause injury to mesophyll cells (b) followed by collapse of adjacent hyphae (a). Scale represents 10 μ .

mycelium died because of starvation or antagonistic activity of the host cells. The mesophyll cells were injured for about 50 μ around the mycelium.

There was no significant difference between the development of mycelium of this collection and the typical ones in Jonathan (fig. 4, F). The mycelium covered a large area and sent runner hyphae slightly farther beyond the hypertrophied tissue than did the typical rust collections. These hyphae appeared to be active since they stained readily with cotton blue, had normal nuclei and their haustoria had penetrated many mesophyll cells.

Class No. 3. The rust collections comprising this class (nos. 24 and 34 of the 1935 experiment and probably other collections of the 1933 experiment) failed to infect either of the more resistant varieties, Northwestern Greening or Delicious (fig. 2, A). The failure of infection on certain varieties has been used sparingly as a differential character since it might represent errors in technique. For instance, collections nos. 4, 13 and 15 on Tolman; 1, 8 and 21 on Turley; 24 on Grimes Golden and 3 on Delicious (1935 experiment) were not separated on this character because not enough leaves of all ages were available for observation. Failure of this particular class to be infective is considered a differential character, because there were several collections that produced the reaction, the two most resistant varieties gave the same reaction, the cultures were very infective on less resistant varieties, and at least eight leaves of different ages were inoculated with each collection.

Although leaf tissue was examined under binoculars and from paraffin sections, no evidence of penetration or establishment of mycelium could be found.

Class No. 4. The collections comprising this class (nos. 5 and 6 of the 1935 experiment) caused a large irregular yellow spot on the variety Turley, which usually supported a single spermogonium in the center. The leaf tissue adjacent to these infection centers became yellowish or necrotic (fig. 1, A and B), and the leaf eventually fell from the tree. Rust collection no. 6 caused six of seven inoculated leaves to fall by June 29, and both nos. 5 and 6 had defoliated their respective branches by July 12. The virulence of this race was attributed, at first, to the large number of sori produced. Leaves with as few as four to eight infection centers, however, were killed, even though leaves with four times as many infection centers by collection no. 3 on an adjacent branch remained firmly attached.

Invasion of the epidermis and palisade layers by collection no. 5 was similar to the typical infection of this variety described above, except that the cells of the palisade layer were less severely injured during the early stages of development. Invasion of the spongy parenchyma, however, was entirely different from the typical infection in which the mycelium did not spread very much. The

virulent race sent slender runner hyphae among the spongy parenchyma cells which underwent hypertrophy and hyperplasia (fig. 4, B). Haustoria had penetrated many of these cells without apparent injury to either host or parasite. After the spermogonia began to mature, a general collapse was initiated in the palisade layer near the point of penetration (fig. 4, C). This injury eventually included the spongy parenchyma and tissue surrounding the spermogonia (fig. 4, D). In sori that exhibited this general destruction of tissue, cells beyond the invaded area were granular and collapsed. Either the necrotic tissue was harmful to the surrounding noninvaded tissue, or the pathogen produced a toxin which diffused beyond the infected area. There was no indication that the mycelium was injured before the host collapsed.

Class No. 5. The collections which comprise this class (nos. 11, 22, 23, 25, 30, 31, 32 and 35 of the 1935 experiment) failed to infect Northwestern Greening but caused typical flecks on Delicious. Although this group may be similar to no. 3, it is not described as such until this reaction has been confirmed by additional observations. In a parallel situation, collections nos. 3 and 17 which failed to infect Delicious, but caused flecking on Northwestern Greening, might also be separated as a class and considered a potential race.

Classes Nos. 6 and 7. These two classes are members of a series identified by the reaction of Ben Davis (fig. 3, A). The infection produced by class 6 (collections nos. 2 and 9) consisted of a definite punctiform fleck rather than a large fleck with diffuse border described for the typical infection. Class 7 (consisting of collections nos. 4, 5, 6 and 7 of the 1935 experiments) was differentiated by its ability to produce spermogonia in most of the flecks. Since these two classes have reactions which intergrade into the typical reaction on Ben Davis, they are not considered to be as distinctive as the first four groups described above. However, the differences in macroscopic symptoms were associated with very definite histological features.

Collection no. 2 of class 6, penetrated the epidermis and in at least one sorus extended laterally for two cells before sending hyphae into the palisade layer. Four or five cells collapsed to either side of the point of penetration into the palisade layer (fig. 6, G) in a fashion comparable to that illustrated for collection no. 15 on Turley (fig. 5, A). Definite remains of inter- and intracellular mycelium were found among the dead host cells, but none of the hyphae penetrated beyond the first layer of palisade cells. It was impossible to determine whether the host or parasite was first to be injured. The final appearance of the sori was similar to that described by Nusbaum (28) for resistant hosts invaded by typical collections.

Collection no. 7 of class 7 penetrated the epidermis and palisade layers as described above for the typical collections. However,

the mycelium which was established in the spongy parenchyma sent out extensive runner hyphae, which instigated pronounced hypertrophy and hyperplasia of the spongy parenchyma cells without causing them to become granular and necrotic. The mycelium developed intercellularly and sent filamentous haustoria into the cells, many of which became hypertrophied into a palisade-like arrangement (fig. 6, H). Spermatogonia developed in these hypertrophied tissues on the under surface of the leaf, a phenomenon rarely observed in the more susceptible varieties. About the time the spermatogonia developed to a mature size, the hypertrophied cells at their bases (fig. 5, F) collapsed. These cells died from the outer edge of the spermatogonia inward, and necrosis was limited to the area around the spermatogonia. The remainder of the sorus was uninjured and the hyphae appeared to be functional. This localized injury that destroyed the spermatogonium was entirely different from that described above for group 4 on Turley, in which there was a general collapse of the invaded tissue after the spermatogonium began to form.

Class No. 8. Collection no. 15 that comprises this class was practically avirulent on Turley since it rarely caused more than a few small restricted yellow flecks (fig. 1, A, no. 15). Very few sori were available for histological study. The mycelium apparently penetrated the epidermis and one layer of palisade cells, but little of the nature of the reaction could be determined. The most extreme type of injury to be observed is illustrated in fig. 5, A. Three or four cells in the upper palisade layer and the adjacent epidermal cells collapsed. What appeared to be an isolated strand of mycelium was identified between the cells in two of the three sori studied.

DISCUSSION

Most of the studies upon physiologic specialization in the rusts have been concerned with the reaction of the uredo- and teleutospore hosts (1, 29, 31). Few workers have investigated the differential reaction of the aecidial hosts, probably because of the seasonal appearance of suitable inoculum and host materials. However, it has been shown that some of the rusts may be specialized for their aecidial hosts (9, 10, 11, 14, 16, 17, 20, 22, 24, 25, 32). In those species, such as *Puccinia graminis*, *P. coronata* Corda and *P. rubigovera* (DC) Wint. where specialization for the alternate hosts has been studied, it has been found that the races specialized for one host may or may not be specialized for the other. For example, *P. graminis* consists of six varieties specialized for different teleutospore hosts, but Levine and Cotter (17) found that all of these except *P. graminis* var. *phlei-pratensis* were capable of infecting the aecidial hosts (*Berberis* spp.). On the other hand, *P. coronata* has been divided into a number of species by European workers (9,

10, 15, 16, 24, 25) because it was specialized for different aecidial and teleutospore hosts. Melhus, Dietz and Willey (22) concluded that the four varieties, which they identified on teleutospore hosts were not specialized for different species of *Rhamnus*. Frazer and Ledingham (11) have attempted to consolidate these two viewpoints by describing four varieties of *P. coronata*, which are specialized for both aecidial and teleutospore hosts. A similar situation exists in the leaf rust of cereals caused by *P. rubigo-vera*. Mains (20) divided the species into a number of series, each specialized for different aecidial hosts and further subdivided each series into varieties and races according to their specialization for different teleutospore hosts. Some of the series are specialized for certain teleutospore hosts as well as aecidial hosts, but others may have a common teleutospore host even though they are specialized for different aecidial hosts.

Physiologic specialization in *Gymnosporangium juniperi-virginianae* has not been studied except for the observation by Bliss (2) and Crowell (7). This may be due to the innate difficulties in obtaining host materials suitable for cross-inoculation, the absence of an uredospore generation that would facilitate repetition of experiments, or the genetical heterogeneity of the inoculum. However, there is no reason why the differential reaction of the aecidial host might not be used just as readily as that of the teleutospore host in identifying races. The only reason the groups described in this bulletin are not designated as definite races is because of the limitations to the experimental techniques employed. However, the type reactions observed were just as distinctive as those employed in cereal rust investigations and could just as well be considered as diagnostic of races.

From these studies and those by Nusbaum (28) the development of *G. juniperi-virginianae* on a susceptible host such as Wealthy may be divided into the following stages: 1. Sporidia germinate on the upper surface of the leaf, 2. a germ tube penetrates the cuticle and epidermis after forming an appressorium, 3. invasive hyphae in the epidermal cells penetrate inter- or intracellularly into the palisade layer, 4. intercellular hyphae develop in the mesophyll, particularly in the spongy parenchyma, 5. cells of the spongy parenchyma show hypertrophy and hyperplasia following penetration by haustoria, 6. hyphae aggregate in the palisade layer to form a cushion which later develops into an erumpent spermogonium, and 7. aecidia develop in the hypertrophied spongy parenchyma. Some of the differences between this normal sequence of events and the infection failures that resulted in several varieties are summarized below.

Class 3 apparently failed to penetrate the epidermis on leaves of the varieties Delicious and York Imperial. It is possible that the germ tubes did not develop sufficient mechanical pressure to

penetrate the cuticle and epidermis of even a young leaf of these varieties. Such failures would be analogous to those observed for typical races on older leaves of York Imperial and Wealthy (12, 13, 23, 28). However, further study is needed on this race, since Nusbbaum (28) has reported that young leaves of highly resistant varieties such as Baldwin show no effective mechanical resistance to penetration. Melander and Craigie (21), on the other hand, have correlated resistance of *Berberis* sp. to sporidia of *P. graminis* with the resistance of the cuticle and epidermis.

The penetrating hyphae and surrounding host cells of the palisade layer collapsed in Turley and Ben Davis inoculated with collections of classes 8 and 6, respectively. The mycelium of class 2 developed slightly farther in York Imperial before it collapsed among the cells of the spongy parenchyma. It was impossible to determine whether host or parasite collapsed first in these three cases, since the lesions were too old at the time of killing and fixing.

Most of the infection failures occurred after the mycelium became established in the mesophyll and had spread into the spongy parenchyma. Sori on Grimes Golden and Maiden Blush contained many depleted hyphae among uninjured host cells. There was little evidence of cell penetration and injury by haustoria in Grimes Golden, but cells of Maiden Blush near the periphery of the sorus had collapsed after penetration by the haustoria. It is postulated that the pathogen must penetrate the spongy parenchyma cells without undue injury and stimulate them to growth before the pathogen can obtain proper nourishment to continue its development. This viewpoint is supported by the observation (28) that partially mature leaves of the normally susceptible Wealthy, which failed to be stimulated by invading hyphae, never supported spermogonia and aecidia of the fungus.

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